Remarks

Reconsideration of this Application is respectfully requested.

I. Status of the Claims

Reconsideration of this Application is respectfully requested.

Claims 1-13, 17, 19-32 and 45-70 are pending in the present application, with claims 1 and 45 being the independent claims.

Based on the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

II. Summary of the Office Action

In the Office Action dated November 23, 2009, the Examiner has made one rejection of the claims. Applicants respectfully offer the following remarks concerning this element of the Office Action.

III. The Rejection Under 35 U.S.C. § 103(a) is Traversed

At pages 3-9 of the Office Action, the Examiner has rejected claims 1-13, 17, 19-32 and 45-70 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Dang *et al.*, June 20, 2003 (U.S. 2003/0119107 A1; hereinafter "Dang") in view of Yan *et al.*, February 2003 (U.S. 2003/0027331; hereinafter "Yan") and Kehat *et al.*, 2001 (The Journal of Clinical Investigation, Vol. 108, No. 3, p. 407-414; hereinafter "Kehat"). Applicants respectfully disagree.

The factors to be considered under 35 U.S.C. § 103(a), are the scope and content of the prior art; the differences between the prior art and the claims at issue; and the level

of ordinary skill in the pertinent art. *See Graham v. John Deere*, 86 S.Ct. 684 (1966) and MPEP §2141. This analysis has been the standard for 40 years, and remains the law today. *See KSR International Co v. Teleflex Inc.*, 127 S.Ct. 1727 (2007). The Office has recently published Examination Guidelines to aid Examiners in formulating obviousness rejections. *See* MPEP § 2141 (hereinafter "the Examination Guidelines"). Seven rationales are suggested by which obviousness may be found, *e.g.*, by combining elements in the art or substituting one known element for another. As a common thread through all the rationales, the Examiner must establish on the record that a person of ordinary skill in the art would have recognized that the results of the combination or substitution were *predictable*. *Id.*

The Examiner has not met the burden of establishing a *prima facie* case of obviousness based on the Examination Guidelines. Specifically, the Examiner has not established that the ordinary artisan reading Dang, Yan and Kehat in combination would arrive at the presently claimed method that uses the rocking of a container containing a liquid single cell suspension culture of a certain amount and concentration of pluripotent cells to generate a high volume and density of embryoid bodies (EBs).

The Examiner asserts that "[i]t would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to rock pluripotent cells in single cell suspension because Dang teaches single cell liquid suspension cultures (scLSC) of ES cells and a bioreactor or culture system that keeps the cells and/or spheroids in liquid suspension by stirring or other means, such as agitation of the system." *See* Office Action at page 6. However, Applicants respectfully contend that the Examiner has cherry-picked various elements from both the prior art and the embodiments disclosed in Dang to reach his conclusions. Specifically, the single cell liquid suspension cultures (scLSC)

discussed in paragraphs [0025] and [0028] as well as Figure 1 are used by Dang to represent "the current technologies used to culture pluripotent stem cells." *See* Dang at page 3, paragraph [0025]. Thus, the scLSC represents the prior art rather than being an embodiment of Dang. Furthermore, as shown in Figure 1, the single cells are singled out and placed in separate containers (*e.g.*, a microtiter plate). Hence, as explained in Dang, the scLSC is a static and batch-style culture and thus not agitated. *See* Dang at page 1, paragraphs [0008] and [0009].

Furthermore, as illustrated in Figure 2, Dang teaches *either* the use of an encapsulated stirred culture (ESC) in a reactor and culture system with an impeller *or* an encapsulated liquid suspension culture (ELSC), which again is a static system. *See also* Dang at page 6, paragraph [0069] and page 9, paragraph [0120] where it is explicitly stated that the ES cell suspension was cultured in "unagitated" petri dishes. Thus, Applicants respectfully assert that it would not have been predictable to one of ordinary skill in the art to agitate the single cell suspension of the ELSC cells of Dang, given that Dang explicitly teaches the single cell suspension to be a static system.

Furthermore, piecing these cherry-picked elements together, is insufficient to establish *prima facie* obviousness, because both the cited art and the claimed invention must be considered *as a whole*. Here, the claimed invention as a whole includes the inventors' discovery that agitation such as rocking of a certain amount and concentration of a single cell suspension of ES cells is superior to the other conventional methods of embryoid body formation. *See* specification at page 9, lines 8-14. As discussed in the specification, the agitation (*e.g.*, rocking) of a single cell suspension of pluripotent cells allows for the generation of EBs in high density which has no negative influence on the differentiation capacity towards different cell types such as cardiomyocytes, neurons,

endothelial cells and liver cells. *See* specification at page 9, lines 14-17. For example, the ES cells are less exposed to shear stress than the conventional spinner flasks or stirring cultures, thus the ability of the cells to differentiate in an appropriate manner is not negatively influenced. *See* specification at page 10, lines 7-9. Accordingly, the method of the present invention is able to generate a large amount and high density of embryoid bodies compared to the conventional methods. *See* specification at page 9, lines 8-10 and page 17, lines 1-4. In contrast, the art cited by the Examiner would not have provided a reasonable expectation of success in obtaining the presently claimed methods because the mere substitution or combination of elements from the three references cited by the Examiner would not have resulted in a predicable method of producing embryoid bodies from a single cell suspension of pluripotent cells, as presently claimed. Thus, Applicants respectfully assert that if the cited art and the claimed invention are properly considered as a whole, the conclusion must be reached that the claimed invention is non-obvious.

Dang is directed toward the improvement of stirred bioreactors and differentiation of cells in stirred cultures by controlling spheroid, EB or cell aggregation by *e.g.*, encapsulation within a matrix. Indeed, as discussed below, Dang does not come even remotely close to the present invention. The experiments of Dang clearly show that efficient EB generation in liquid culture is only possible either under static conditions, *i.e.*, non-agitation of the system at low concentrations of ES cells/ml or where cell aggregation is effectively controlled by encapsulation. Specifically, the description of Dang as a whole makes it abundantly clear that only encapsulation of the ES cells enables the use of stirred cultures for the generation of EBs by preventing of aggregation between separate EBs. *See* Dang, *e.g.*, at Example 2, paragraph [0161] and Fig. 2.

This is the actual teaching and background against which paragraph [0053] of Dang needs to be interpreted. Applicants respectfully assert that reading Dang as a whole, it would be clear to a person of ordinary skill in the art that the statement that in addition to stirring "other methods or means can be used, such as by agitation of the system," requires that further conditions, in particular encapsulation, must be used in order to achieve the goal of Dang.

As discussed in the Amendment and Reply Under 1.114, filed October 26, 2009, the fact that the teaching of Dang concerning the preparation of EBs is confined to the use of stirred cultures only after encapsulation of the ES cells is further corroborated by the inventors and applicants of Dang. For example, in the reply to a Communication pursuant to Article 96(2) EPC (Exhibit 1) issued by the European Patent Office on January 19, 2005 in European patent application No. 02 745 012.1, which is the corresponding European application to the Dang reference, the Applicants state at page 2, first full paragraph that "the encapsulation technology of the present invention solves the problem, enabling the use of stirred cultures without EB interaction,...." Further, at page 3 of the reply, emphasis is put on the use of stirred suspension cultures and stirred bioreactors, respectively. In support of its contention, the reply is accompanied by two publications from the inventors of the Dang reference, which emphasize the use of stirred suspension by reactors. *See* Dang *et al.*, *Stem Cells 22*: 275-282 (2004) in the abstract (Exhibit 2) and Bauwens *et al.*, *Biotechnology and Bioengineering 90*: 452-461 (2005) (Exhibit 3). In this context, the Examiner is kindly referred to the abstract of Exhibit 2 (left column, third sentence) which states that "[a]ggregation between EBs (agglomeration), however, inhibits cell growth and differentiation in stirred or high-cell-density static cultures" and the last sentence of the abstract referring to the use of stirred-

suspension bioreactors. As can be inferred from these statements, according to the inventors of Dang only two options for the aggregation of EBs existed at the time, *i.e.*, (1) static cultures such as hanging drop and methylcellulose cultures and (2) stirred cultures which is consistent with the teaching of Dang. *See* Dang at paragraphs [0008] to [0013].

As discussed in the specification, the method of the present invention is superior to the stirring method (*e.g.*, spinner flask technology) for generating embryoid bodies. Specifically, as indicated above, compared to cultures in spinner flasks, in the agitation method (*e.g.*, rocking) the ES cells are much less exposed to shear stress, whereby the capability of the cells to differentiate in an appropriate manner is not negatively influenced. *See* specification at page 10, lines 7-9. In addition, the specification points out that in previous methods for the production of embryoid bodies the yield of embryoid bodies was in the range of 50/ml. However, using the present method, one of ordinary skill in the art could obtain embryoid bodies generally in the range of 500/ml. *See* specification at page 17, lines 1-5. Thus, these results confirm that the agitation method of the present invention is superior to the stirring method for generating embryoid bodies.

In maintaining the rejection under 35 U.S.C. §103(a), the Office failed to properly consider the Declaration Under 37 C.F.R. § 1.132 of Dr. Silke Schwengberg, filed on October 26, 2009 (hereinafter "the Schwengberg Declaration"). The Office "must give the declaration[] meaningful consideration before arriving at its conclusion." *See In re Sullivan* 498 F.3d 1345, 1353. (Fed. Cir. 2007). However, in this case, the Examiner has provided no analysis for disregarding the data presented in the Schwengberg Declaration.

In *In re Sullivan*, the Federal Circuit firmly established that the Office cannot simply disregard an Applicant's rebuttal evidence such as a Declaration without proper consideration. *Id.* In *In re Sullivan*, the claimed Fab fragments to neutralize rattlesnake venom were rejected as being obvious. *Id.* at 1348-1350. The Applicants presented three Declarations during examination of the application in an attempt to rebut the rejection.. *Id.* at 1352-1353. The Board, however, failed to consider the Declarations. *Id.* Upon this failure, the Federal Circuit vacated and remanded the Board's decision and held that "the Board must give the declarations meaningful consideration before arriving at its conclusion." *Id.* at 1353.

Similarly, the Examiner has failed to meaningfully consider the data presented in the Schwengberg Declaration. Specifically, as discussed in the Schwengberg Declaration, the agitation method of the present invention was compared to the stirring method found in the art. *See* the Schwengberg Declaration at paragraphs 9-12. The agitation method produced a higher (i) yield of EB per ml and (ii) yield of differentiated cells (*e.g.*, cardiac cells) per ES cell originally seeded into the differentiation culture compared to the stirring method. *See* the Schwengberg Declaration at paragraphs 9-13. Therefore, Applicants respectfully request that the Examiner give full consideration to the Declaration.

Dang does not teach or suggest that rocking of a single cell suspension culture of pluripotent cells would produce a high volume and density of embryoid bodies. Dang only suggests methods of improving the already known stirring method which as indicated above is inferior to the method of the present invention. Furthermore, the single cell liquid suspension culture (scLSC) discussed in Dang represents the prior art rather than being an embodiment of Dang. Thus, based on the disclosure of Dang, one of

ordinary skill in the art would not have predicted that rocking of a single cell suspension of pluripotent cells would generate a high volume and density of EBs.

The deficiencies of Dang are not cured by the disclosure of Yan and Kehat. While Yan teaches using a single cell suspension to generate embryoid bodies, Yan does not suggest or teach a method for generating embryoid bodies by rocking a single cell suspension of pluripotent cells. The method used in Yan to generate the embryoid bodies is a static culture. Nowhere in Yan is it taught or suggested that a single cell suspension culture should be rocked in order to produce a high volume and density of EBs. Accordingly, one of ordinary skill in the art would not have predicted based on Yan that rocking of a single cell suspension of pluripotent cells would generate a high volume and density of EBs.

Kehat teaches using **clumps** of ES to generate EBs, not a **single cell** suspension, as claimed. *See* Kehat at page 408, the paragraph bridging left and right column. Furthermore, like Yan, the culture taught in Kehat is a static culture. *See* Fig. 1 of Kehat at page 408 and the figure legend. Nowhere in Kehat is it taught or suggested that a single cell suspension culture should be rocked in order to produce a high volume and density of EBs. Accordingly, one of ordinary skill in the art would not have predicted based on Kehat that rocking of a single cell suspension of pluripotent cells would generate a high volume and density of EBs.

None of the references cited by the Examiner teach a method of producing embryoid bodies from pluripotent cells by rocking a single cell suspension with a concentration of $0.5x\ 10^6$ to $5x\ 10^5$ cells/ml or $0.1x\ 10^6$ to $1x\ 10^6$ cells/ml. The mere substitution or combination of elements from the combination of references cited by the Examiner would not have resulted in a predicable method of producing embryoid bodies from

pluripotent cells, as presently claimed. Thus, for at least the foregoing reasons, Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness as set out in the Examination Guidelines and respectfully request that this rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

IV. Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Shannon A. Carroll, Ph.D.
Attorney for Applicants
Registration No. 58,240

Date: February 23, 2010

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

1075195_1.DOC